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March 27, 2003

Christine Todd Whitman, Administrator
U.S. Environmental Protection Agency
Ariel Rios Building (1101A)
1200 Pennsylvania Ave., NW
Washington, DC 20460

Re: Comments on Lubrizol Corporation's Test Plan for Petroleum Oxidates

Dear Administrator Whitman:

The following comments on the High Production Volume (HPV) Challenge test plan for Petroleum Oxidates and Derivatives, submitted by the Lubrizol Corp., are submitted on behalf of People for the Ethical Treatment of Animals, the Physicians Committee for Responsible Medicine, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These health, animal, and environmental protection organizations have a combined membership of more than ten million Americans.

Lubrizol's test plan for oxidized petroleum compounds includes eight substances, in two subcategories. These substances are prepared by controlled, liquid-phase, partial oxidation of petrolatum, slack wax, and petroleum distillates, using atmospheric air. The list of substances is almost identical to that in the American Petroleum Institute's recent waxes category. The derivatives of these substances are then prepared by a second process, methyl-esterification. We commend the establishment of this category, but contend that its members should be included in the API's recent Waxes and Related Materials Category test plan (http://www.epa.gov/chemrtk/wxrelmat/c13902tc.htm).

We agree with Lubrizol's inclusion of both lighter and heavier hydrocarbon products in a single category and its identification of the lighter-molecular-weight members of the category as having a bounding toxicity (p. 10). We have long supported this approach in our comments on other test plans. By incorporating a broader category, a more global view of the toxicity of similar chemicals can be obtained, enabling hazard assessment without additional animal testing.

As stated in the test plan, the final product is often 50% unreacted starting material, and most of this starting material for subcategory 2 has already been reviewed in API's Waxes and Related Materials Category. All substances in the category are actually complex mixtures of derivatized hydrocarbon compounds—the toxicity of many of these compounds is either already characterized or can be extrapolated through structure-activity relationships. Importantly, the overwhelming theme of the existing data of both API's Waxes test plan and this test plan is the low toxicity of these compounds due to their high molecular weight, low water solubility, high $K_{o/w}$, and low overall bioavailability. Furthermore, high molecular weight ester compounds have been generally found to have low toxicities (see, for example, The Pine Chemicals Association Rosin Esters test plan and The Flavor and Fragrance Consortium's Terpenoid Tertiary Alcohols and Related Esters Category, to name two general groups of low-toxicity esters). Providing additional information with more detailed compositional information on these products could

support greater use of a larger category. Lubrizol tacitly acknowledges the role of these physicochemical factors in affecting toxicity, as it selects the compound with the lightest molecular weight and highest water solubility for additional testing, which "indicates that it will be more bioavailable than the other members of this category" (p. 10).

We support the formation of a scientifically valid category with a number of substances. However we would like to know why Lubrizol has not combined its category with the API category mentioned above. We are, of course, very concerned about the remaining proposed testing on animals, which includes the following:

1. Acute fish toxicity test;

2. Acute mammalian toxicity test; and

3. Combined repeat-dose/reproductive/developmental study (OECD No. 422).

These tests will result in the deaths of approximately 800 animals.

With respect to fish toxicity, the EPA has stated that acute fish tests are inappropriate for compounds with log $K_{o/w}$ values above 4.2, and recommends that with such highly hydrophobic compounds a chronic *Daphnia* test be used instead of acute fish and *Daphnia* tests (EPA 2000, p. 81695). The log $K_{o/w}$ value of subcategory 1 is estimated to be 3.3-7.06, and that of subcategory 2 is estimated to be over 4.9 (test plan, p. 5). The fish test should therefore not be carried out.

An additional reason why the fish test is unnecessary is connected with ecotoxicity and mammalian toxicity tests having different purposes. The mammalian toxicity tests are assumed to be useful for predicting toxicity in individual humans. Fish tests, on the other hand, are not for predicting toxicity in individual fish, but for predicting economic loss (to commercial and "sport" fisheries) and ecologic damage (fish are an important part of the food chain). The fish test therefore aims to show whether exposure with petroleum oxidates and derivatives will result in large-scale fish death. However, water pollution can wipe out fish stocks even with no direct toxicity, because killing the food of the fish will lead to starvation. Carps and catfishes are herbivorous, eating mostly algae, whereas most other familiar North American freshwater fish species are carnivorous, eating worms, small crustaceans, smaller fish, insect larvae, etc. However, the toxicity of petroleum oxidates and derivatives towards these types of organism is unknown, as shown by the inclusion in the test plan of tests on aquatic invertebrates and algae (p. 6). Fish tests should not be carried out while other types of aquatic toxicity are uncertain.

As far as the proposed mammalian tests are concerned, the plans are premature in the context of the present paucity of data on analytical chemistry (mentioned above) as well the lack of exposure information. With respect to the latter, large numbers of people are exposed to petroleum oxidates and derivatives, with the numbers in the cases of some of the substances approaching 100,000 people per year (NIOSH 1981-1983). However, nothing is known about the duration, concentration, etc., of the exposure, and exposure evaluations are therefore essential. Epidemiology studies are also appropriate, and would probably provide far more information than animal studies.

Finally, if Lubrizol does wish to carry out the tests indicated in the test plan, there is a range of *in vitro* and *in silico* alternatives to fish and acute and reproductive/developmental mammalian toxicity tests that should be considered:

(i) *In silico fish test substitute*. Quantitative structure activity relationship (QSAR) programs provide *in silico* methods for estimating toxicity to fish and other aquatic organisms. The EPA itself encourages the use of one established QSAR: ECOSAR (EPA 2002).

(ii) *In vitro fish test substitute 1*. TETRATOX is an assay based on the protozoan *Tetrahymena pyriformis* (Larsen 1997). With 50% growth impairment as the endpoint, the results of this assay show close similarity to toxicity in the fathead minnow (Schultz 1997), and the extensive available information demonstrates that TETRATOX is an effective alternative to fish testing. It is in fact already used extensively in industry, and is being considered for regulatory acceptance by the OECD. It is also rapid, easy to use, and inexpensive. On October 23, 2001, PETA and PCRM held a meeting with EPA to facilitate incorporation of an *in vitro* aquatic toxicity test into the HPV program, and Dr. Schultz (Professor of Predictive Toxicology, University of Tennessee College of Veterinary Medicine) made a presentation about TETRATOX. On December 5, 2001, PCRM scientist Nicole Cardello presented the details of this meeting, and our proposal, in a letter to EPA Assistant Administrator Stephen Johnson. After more than one year, there has still been no response from Mr. Johnson or anyone else in the agency. We again request a thoughtful, scientific and specific reply to this letter. It is the stated goal of the EPA to incorporate *in vitro* methods into the HPV program, and this presents an ideal opportunity for action rather than words.

(iii) *In vitro fish test substitute 2*. The test protocol and performance parameters of the recently validated *Dar*T test are described in detail in Schulte (1994) and Nagel (1998). Briefly, however, it uses fertilized zebrafish (*Danio rerio*) eggs as a surrogate for living fish. The exposure period is 48 hours, and assessed endpoints include coagulation, blastula development, gastrulation, termination of gastrulation, development of somites, movement, tail extension, eye development, circulation, heart rate, pigmentation and edema. Endpoints comparable to *in vivo* lethality include failure to complete gastrulation after 12 hours, absence of somites after 16 hours, absence of heartbeat after 48 hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of test substances. The reliability and relevance of the *Dar*T test have recently been confirmed in an international validation study coordinated and financed by the German Environmental Protection Agency, and predictions of acute toxicity from the *Dar*T test were highly concordant with *in vivo* reference data (Schulte 1996). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius 1995), and is clearly suitable for immediate use as a replacement for the use of fish in the HPV program's screening-level toxicity studies.

(i) *Mammalian acute toxicity test substitute*. We urge Lubrizol to discuss with the EPA the possibility of using the basal cytotoxicity instead of the *in vivo* acute toxicity test. In the Multicentre Evaluation of *In Vitro* Cytotoxicity, a worldwide study organized by the Scandinavian Society for Cell Toxicology, basal cytotoxicity assays were found to be more reliable predictors of human lethal doses, for 50 reference chemicals, than were rodent LD_{50} values. Furthermore, when certain other human toxicokinetic data (blood-brain barrier passage; timing of lethal action, etc.) were used in conjunction with the cytotoxicity results, the prediction of human lethal concentrations improved markedly (Clemedson 1996a, 1996b, 1998a, 1998b, 2000, Ekwall 1998a, 1998b, 2000). The assay used involves measuring the effect of compounds on the viability of human basal keratinocytes, which is determined from the intensity of staining by neutral red, a dye that is taken up by healthy cells more than by dead and low-viability cells. At the very least, the *in vitro* cytotoxicity test must be used *prior to* proceeding with any *in vivo* acute toxicity testing per EPA guidance found at http://www.epa.gov/chemrtk/toxprtow.htm.

(ii) *Mammalian reproductive/developmental toxicity screening test*. *In vivo* developmental and reproductive toxicity tests have not been validated for humans. However, an *in vitro* embryotoxicity (a key endpoint in developmental toxicity) test method, the rodent Embryonic Stem Cell Test (EST), has recently been validated by the European Centre for the Validation of Alternative Methods, and the ECVAM Scientific Advisory Committee has concluded that this test is ready to be considered for regulatory purposes (Genschow 2002). If a positive result is found in the EST, the substances should be treated as developmental toxicants/teratogens, and no further testing should be carried out within this screening-level program. Although we have written to the EPA repeatedly concerning the inclusion of the EST in the HPV Program, with correspondence dating back more than six months, we have received no reply. We urge Lubrizol to correspond directly with the EPA on the incorporation of this validated non-animal test.

We would greatly appreciate receiving a direct response to our concerns. I can be reached at 757-622- 7382, ext.1304, or via e-mail at JessicaS@peta.org.

Sincerely,

Jessica Sandler
Federal Agency Liaison

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